American Journal of Hematology 59:302–308 (1998)

IgM Myeloma: Case Report With Immunophenotypic Profile

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Immunoglobulin (Ig)M myeloma is a distinct subtype of multiple myeloma (MM) displaying clinical and pathologic features of both MM and Waldenström's macroglobulinemia (WM). Although the immunophenotypic characteristics of classic MM and WM have been reported, the surface antigen expression of IgM myeloma has not been reported. We report a case of IgM myeloma and describe its immunophenotypic profile using flow cytometry. The cells showed a hybrid MM-WM phenotype, strongly expressing CD38 but lacking CD45 and DR, typical for plasma cells; however, pan-B cell antigens CD20 and FMC7 as well as weak monoclonal surface Ig also were positive, resembling B-cell lymphoproliferative malignancies. Discordant B-cell antigen expression was present, in that pan-B antigens CD19 and CD22 were absent. In addition, B-cell activation antigen CD23, early B-precursor antigen CD10, and pan-T antigen CD5 were not expressed. Although CD20 and weak surface Ig expression have been reported in MM, FMC7 positivity has not been seen. The data therefore suggest that IgM myeloma may have a unique phenotype with characteristics of both MM and WM. Am. J. Hematol. 59:302–308, 1998.

Key words: multiple myeloma; Waldenström's macroglobulinemia; lymphoproliferative disorders; IgM; flow cytometry

INTRODUCTION

The distinction between multiple myeloma (MM) and Waldenström's macroglobulinemia (WM) usually is straightforward and poses no diagnostic dilemma. The presence of multiple osteolytic lesions (often symptomatic), nonimmunoglobulin (Ig)M monoclonal gammopathy and plasma cell infiltration of the bone marrow is consistent with a diagnosis of multiple myeloma [1]. On the other hand, IgM monoclonal gammopathy, lymphadenopathy, hepatosplenomegaly, anemia, and hyperviscosity syndrome in conjunction with a monoclonal lymphoplasmacytoid proliferation in both the bone marrow and peripheral blood is characteristic of WM [2]. However, cases of IgM myeloma have been described, with clinicopathologic features intermediate to those of MM and WM. These patients have a high incidence of lytic bone lesions, decreased IgG and IgA levels, renal failure, hypercalcemia, and Bence Jones proteinuria, common findings in MM; yet, findings typical of WM also occur, including hyperviscosity symptoms, lymphadenopathy, and hepatosplenomegaly [3].

Thus, the possibility of IgM myeloma arises when a patient presents with monoclonal IgM protein and bone lesions, particularly when the characteristic clinical features of WM are lacking. In such cases, the distinction between the two disorders rests on the histological finding of a lymphoplasmacytoid proliferation in WM as opposed to the predominantly plasma cell rich infiltrate in myeloma. If the light microscopic findings are inconclusive in distinguishing the two disorders, then immunophenotyping might prove helpful in resolving the diagnostic difficulty. We present a case of a patient with an IgM monoclonal gammopathy in conjunction with osseous lesions. Morphologic and clinical features were consistent with the diagnosis of IgM myeloma. Flow cytometry revealed an anomalous surface antigen profile, sug-

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Received for publication 22 December 1997; Accepted 12 August 1998

gesting that IgM myeloma phenotypically is an intermediate form between classic MM and WM.

CASE REPORT

The patient is an 83-year-old woman with a history of sequential, spontaneous fractures in the left ribs and in the lumbar spine approximately nine months prior to admission, now complaining of progressive low back pain. Pertinent laboratory results at initial evaluation included: hemoglobin, 8.7 g/dL; hematocrit, 26%; mean corpuscular volume (MCV), 93 fL; white blood cells (WBC), 8.7×10^9/L; absolute neutrophils, 1.2×10^9/L; absolute lymphocytes, 4.1×10^9/L; platelets, 261×10^9/L, blood urea nitrogen (BUN), 25 mg/dL; creatinine, 1 mg/dL; calcium, 10.8 mg/dL; and erythrocyte sedimentation rate (ESR), 130 mm/hr. A monoclonal spike of 2.9 g/dL was present in the gamma region on protein electrophoresis. Subsequent protein studies (by nephelometry) revealed an IgM of 4.8 g/dL (reference range: 60–300 mg/dL) with normal levels of IgA and IgG. The beta-2-microglobulin was 2.08 mg/L (reference range: <3.5) and serum viscosity was elevated at 2.08 mPa.S (reference range: <1.60). Urine immunoelectrophoresis documented a monoclonal kappa light chain by immunoelectrophoresis, with a 24 hr urine protein excretion of 4 g.

Bone films showed compression fractures of T12, L1, L3, and L3. A nuclear bone scan demonstrated increased uptake at the left fifth, seventh, eighth, and eleventh ribs, as well as T11, L1, and L2. Computed tomography (CT) of the chest and abdomen showed no evidence of hepatosplenomegaly or lymphadenopathy. Because of the lack of lymphadenopathy and the presence of multiple osseous lesions, a diagnosis of IgM myeloma was favored. The bone marrow aspirate morphology showed infiltration with small, atypical plasma cells (further described in the Results section), concurring with the clinical impression of IgM myeloma. The patient was treated with a myeloma protocol, which included monthly pamidronate, two cycles of vincristine, doxorubicin, and Decadron infusion, followed by oral melphalan and prednisone for nine months. A significant decrease in IgM to 0.9 g/dL occurred, and serum protein electrophoresis showed only an equivocal monoclonal spike. the course was complicated six months into treatment by a right hip fracture requiring open reduction. At this writing, the patient is fifteen months postdiagnosis. She is ambulatory and capable of all self-care and is not receiving cytoreductive agents.

MATERIALS AND METHODS

Microscopic Examination

Smears prepared from the bone marrow aspirate were stained with Wright-Giemsa. An aspirate clot section was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. In addition, mononuclear cells were isolated from the bone marow aspirate by density gradient separation on Ficoll Hypaque (Pharmacia LKB Biotechnology Inc., Piscataway, NJ). Cytocentrifuge slides prepared from this cell suspension were stained with Wright-Giemsa for cytomorphologic evaluation. Peripheral blood smears were not available for examination.

Immunologic Methods

Immunohistochemical stains were performed on the clot section using a modified biotin-streptavidin method (Ventana Medical Systems Inc., Tucson, AZ). Serial sections of two microns were deparaffinized in xylene and hydrated in a graded series of alcohol solutions. The sections were pretreated by microwave procedure in citric acid buffer (10 mM, pH 6.0) for 10 min. Endogenous peroxidases was blocked by preincubation with 1% hydrogen peroxide in phosphate-buffered saline. Antibodies used included those against kappa (1:1,600 dilution) and lambda (1:6,400 dilution) light chains (Dako Corp., Carpinteria, CA), as well as CD20 (Dako Corp.), CD 43 (Becton Dickinson, San Jose, CA), VS38 (gift from David Y. Mason), and CD45RB (Dako Corp.). Cytocentrifuged slides were stained for Ig using an avidin-biotin alkaline phosphatase method. Slides were fixed for 30 sec at room temperature with formalin-methanol-acetone (1:4:35) and washed with Tris-buffered saline (TBS). Slides were incubated for 15 min sequentially with biotinylated horse antimouse antibody (Vector Laboratories, Burlingame, CA), streptavidin-alkaline phosphatase (Dako Corp.), and alkaline phosphatase substrate-chromogen reagent (Dako Corp.). All incubations were performed at room temperature, and TBS washes followed each incubation step.

Flow cytometry was performed on the FACScan (Becton-Dickinson) using three-color direct immunofluorescence. The surface antigen complement of the Ficoll-isolated bone marrow cells was characterized with commercially available fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated monoclonal antibodies (MoAb), including anti-CD10-FITC, anti-CD19-PE, anti-CD20-PE, anti-CD23-PE, anti-CD3-FITC, anti-CD5-FITC, anti-kappa-FITC, and anti-lambda-PE (Becton Dickinson); anti-CD22-PE, anti-CD38-FITC, and anti-FMC7-FITC (Coulter/Immunotech, Hialeah, FL). The percent reactivity was determined by gated analysis of population clusters identified on the plot of CD45-PECy5 (Caltag, Burlingame, CA) vs. right angle light scatter (side scatter). The percent of events reactive with each MoAb in the analysis gates was determined, setting positive thresholds with isotypic controls.

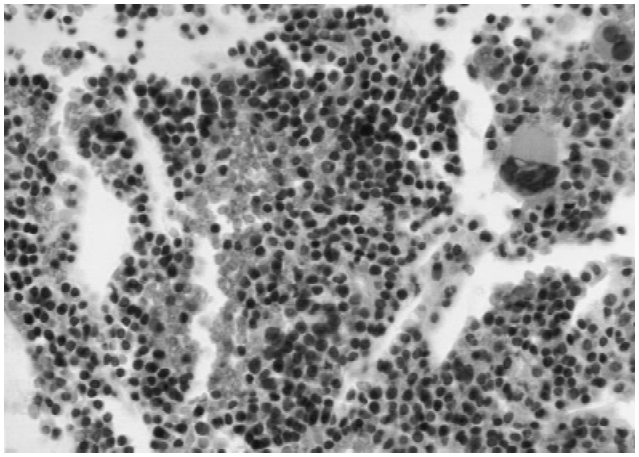


Fig. 1. Bone marrow aspirate clot section showing a monomorphic population of small mononuclear cells with condensed nuclear chromatin. Although most cells resemble mature lymphocytes, some appear plasmacytoid with an eccentrically placed nucleus (H&E, ×100).

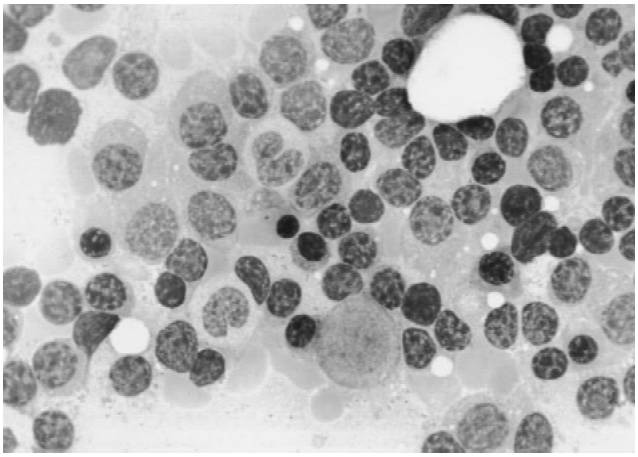


Fig. 2. Bone marrow aspirate showing a predominance of naked nuclei with condensed chromatin admixed with small plasma cells (Wright-Giemsa, ×313).

RESULTS

Light Microscopy

Examination of the bone marrow aspirate clot section revealed a hypercellular marrow (approximately 60%). A monomorphic population of hematopoietic cells occupied the majority of the marrow cellularity (Fig. 1). A small portion of these atypical cells were clearly plasma cells, with eccentrically placed nuclei and margination of nuclear chromatin. However, the distinction between lymphoid cells and atypical plasma cells was quite difficult for the majority of cells comprising this infiltrate. Likewise, the morphology of the Wright-Giemsa stained bone marrow aspirate was problematic. Whereas some small plasma cells were evident, the majority of the slide was composed of naked nuclei with condensed chromatin (Fig. 2). Fortunately, the cytocentrifuge slide of the Ficoll isolate revealed numerous intact small plasma cells (Fig. 3). A 100-cell differential performed on this slide identified 35% small lymphocytes, 2% monocytes, 38% total granulocytes, and 24% atypical plasma cells. The eccentrically placed nuclei in these plasma cells showed condensed, ''ropey'' chromatin with prominent parachromatin spaces. Some plasma cells exhibited a markedly irregular (clover-leaf) nuclear contour or had a small single nucleolus. The abundant cytoplasm was lightly basophilic and lacked prominent Golgi regions. In some cells, the cytoplasm appeared to be packed with tiny, pale, tubular channels or canalicular structures (Fig. 4). This light microscopic finding may correspond to previous descriptions of abundant dilated rough endoplasmic reticulum in the plasma cells of IgM myeloma [3,4].

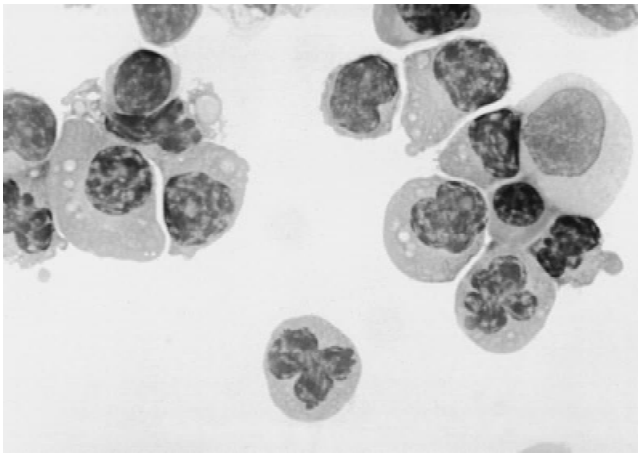


Fig. 3. Ficoll preparation of the bone marrow aspirate reveals many intact, atypical plasma cells, small with irregular nuclear contour, ropey condensed chromatin, and a small nucleolus (Wright-Giemsa, ×373).

Flow Cytometry

Two distinct cell clusters were noted in the CD45/side scatter plot (R1 and R2 in Fig. 5A), and were gated for analysis. Approximately 10% of the collected events resided in a cluster strongly positive for CD45, with weak side scatter, the expected location for lymphoid cells (R1 in Fig. 5A). This cluster showed concordant expression of all pan-B markers, with polyclonal surface light chain (Table I, Fig. 5B). Strong expression of CD38 was not seen (Fig. 5C). Approximately 50% of the collected events were CD45 negative, with weak side scatter (R2 in Fig. 5A); cells that may reside in this location include unlysed erythrocytes, nucleated red cells, and plasma cells. This cluster showed a population of B-cells with discordant antigen expression (Table I, Fig. 5D,E,F).

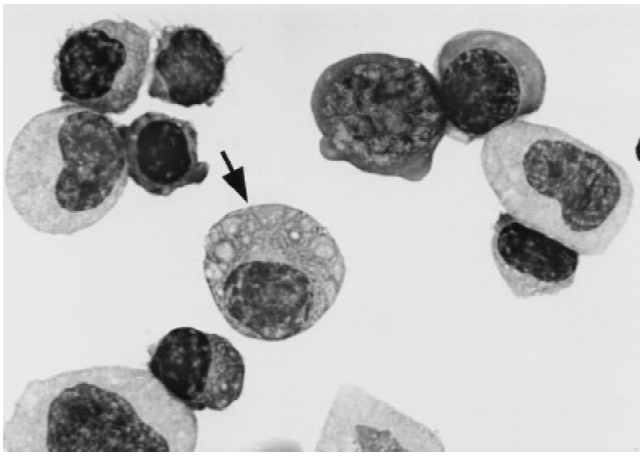


Fig. 4. High power magnification of an atypical plasma cell (arrow). The cytoplasm is packed with pale, canalicular structures (Wright-Giemsa, ×373).

Pan-B antigens CD20 and FMC7 were present, whereas CD19 and CD22 were absent. The B-cells were weakly kappa monoclonal. B-cell activation antigen CD23, early B-precursor antigen CD10, and pan-T antigen CD5 were not expressed. As expected for plasma cells, CD38 was strongly expressed.

Slide Immunophenotyping

Immunoperoxidase studies of the clot section revealed positive staining of the atypical cells with CD20, CD43, and VS38 and negative staining with CD45. The kappa-to-lambda ratio was greater than 16:1. The cytocentrifuge slides exhibited plasmacytoid cells that stained strongly for mu (Fig. 6) but were negative for gamma and alpha heavy chains. Given the weak surface reaction seen with flow cytometry, these results imply the presence of large amounts of cytoplasmic Ig.

DISCUSSION

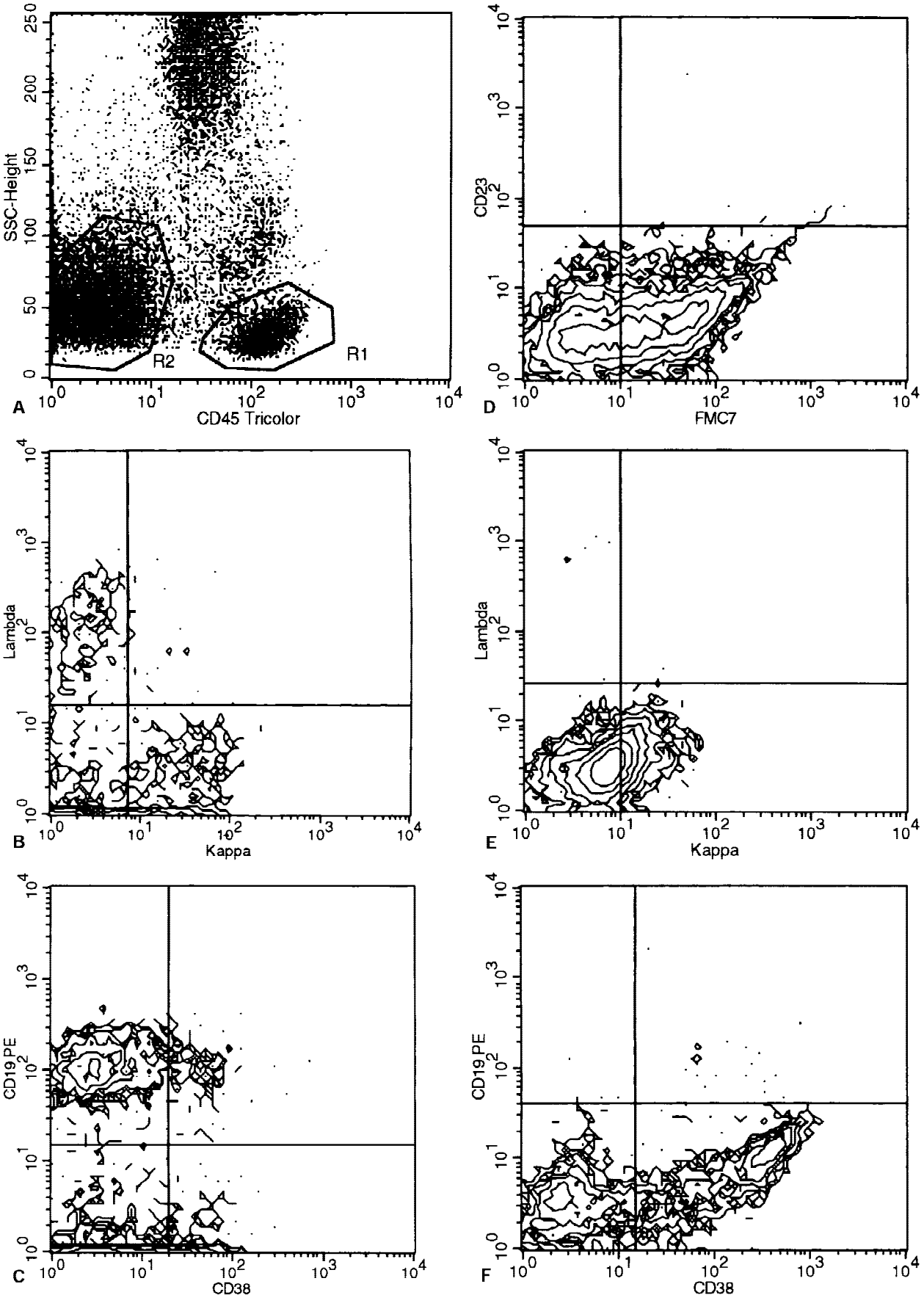
IgM monoclonal gammopathy has been associated with a wide variety of benign as well as malignant lymphoproliferative conditions [5], with large amounts of this paraprotein primarily seen in WM. Although MM is seldom associated with IgM monoclonal gammopathy, cases of IgM myeloma have been reported [3,4,6,7]. The biologic behavior of IgM myeloma seems to more closely resemble that of MM rather than WM, in that the tumor tends to behave in an aggressive manner [3,4,6,7]. Additionally, as was the case with our patient, IgM myeloma may be responsive to chemotherapeutic regimens used for MM, but resistant to the milder forms of chemotherapy used for WM [6,7].

Accordingly, it is necessary to differentiate IgM myeloma from WM. However, clinical criteria are not always helpful. Although the presence of lymphadenopathy and hepatosplenomegaly classically are associated with WM, these clinical features are seen in only 20–40% of cases of WM [8]. Furthermore, Takahashi et al. [4] describe lymphadenopathy and hepatosplenomegaly in approximately 25% of cases diagnosed as IgM myeloma. The presence of osteolytic lesions has been a criterion used by many authors to differentiate MM (including IgM myeloma) from WM [9]. However, many reports associate WM with various types of osseous lesions [6,10–14]. In fact, Moulopoulos et al. [14] observe that 21 of 23 patients with WM had bone involvement as detected by MR imaging. Therefore, IgM myeloma and WM may have overlapping clinical features, and clinical criteria alone should not be used to establish the diagnosis.

When clinical findings are ambiguous, Berman [6] recommends morphologic criteria as the best way of distinguishing between the two disorders; in WM the infiltrate is primarily lymphocytic or lymphoplasmacytic with a minority of plasma cells, whereas in IgM myeloma, the neoplastic cells are predominantly plasma cells [6]. In addition, the plasma cells in IgM myeloma may display a unique morphology. The atypical plasma cells in this case were small, showed condensed nuclear chromatin, lacked perinuclear Golgi zones, and had peculiar canalicular cytoplasmic inclusions in some of the cells, which may represent cysternally dilated rough endoplasmic reticulum. Many of these morphologic features have been reported in other cases of IgM myeloma [3,4].

In cases where the light microscopic examination is equivocal, as was initially the case for our patient, surface marker studies may be helpful in distinguishing IgM myeloma from WM. Both small, mature lymphocytes and well-differentiated plasma cells were present in this

Fig. 5. A: CD45/side scatter plot showing two distinct clusters. R1 corresponds to a population cluster with strong CD45 and low side scatter, presumably lymphocytes, whereas R2 corresponds to a population cluster with weak to negative CD45 and low side scatter, presumably plasma cells B: Kappa-FITC/Lambda-PE plot for the lymphoid (R1) analysis gate showing polyclonal surface light chain expression. C: CD38-FITC/CD19-PE plot for the lymphoid (R1) analysis gate showing minimal weak CD38 reactivity. D: FMC7-FITC/CD23-PE plot for the plasma cell (R2) analysis gate illustrating FMC7 expression and lack of CD23 expression. E: Kappa-FITC/Lambda-PE plot for the plasma cell (R2) analysis gate revealing weak kappa monoclonality. F: CD38-FITC/CD19-PE plot for the plasma cell (R2) analysis gate depicting strong CD38 expression and lack of CD19.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F
CD19 PE
CD38

Fig. 5.

TABLE I. Flow Cytometric Results for Ficolled Bone Marrow Aspirate

Antibody	Strong CD45/low SSC (R1)[a] (%)	Weak-Neg CD45/low SSC (R2)[a] (%)
CD10	1	<1
CD19	27	<1
CD20	30	76
CD22	23	<1
FMC7	22	55
CD23	13	<1
Kappa	16	52 weak
Lambda	8	<1
CD5/CD22	3	<1
CD38	24 weak	64 strong
CD5	65	13
CD3	66	1

[a]Analysis gates as shown in Fig. 5A.

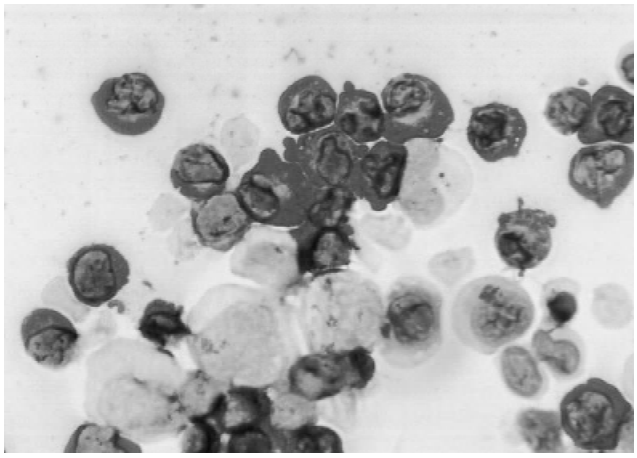

Fig. 6. Immunophenotyping of the cytocentrifuge slides prepared from the Ficolled bone marrow aspirate shows strong cytoplasmic staining for mu heavy chain, evident by the dark stain in the cytoplasm of the positive cells (avidin alkaline phosphatase immunohistochemistry, ×250).

patient's bone marrow. The lymphocytes were predominantly T-cells, and the smaller population of B-lymphocytes displayed concordant expression of pan-B antigens with polyclonal surface Ig. Thus the plasma cells were the malignant component. We demonstrated a unique hybrid phenotype for these plasma cells, with antigen expression typical of plasma cells (CD45 negative, CD38 strongly positive, cytoplasmic Ig strongly positive) combined with discordant expression of B-cell markers (CD20, FMC7, weak surface kappa positive, CD19, CD22 negative). The plasma cells in this case of IgM myeloma are distinctly different from the phenotype of the malignant cells in WM, which typically are positive for pan-B antigens CD19, CD20, CD22, and FMC7 with strong surface and cytoplasmic Ig expression and variable expression of CD38 and CD5 [15–17]. Although B-cell antigens, such as CD20 and CD10 have been reported in non-IgM MM [18–22], to our knowledge, FMC7 has not been described in malignant plasma cells [15,23,24].

In conclusion, the immunophenotypic findings suggest that IgM myeloma is an intermediate form between WM and classical MM. This distinctive hybrid immunophenotype as well as the appearance of the atypical plasma cells can aid in distinguishing IgM myeloma from WM.

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